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RECOVERY DYNAMICS IN MASTER AND AGED ATHLETES

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RECOVERY DYNAMICS IN MASTER AND AGED ATHLETES

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Abstract

A Literature Review of Recovery Processes for Master and Aged Athletes

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Increasing number of masters and aged athletes participate in athletic events each year with increased proficiency and improved performances, yet there are significant physiological differences in the recovery process compared with younger athletes that need elucidation. For example, there are subtle recovery impairments compared to younger athletes amplified by several factors, including increased age-related detraining and sedentary behaviors affecting the training status of aged athletes, female hormonal changes affecting measurement and interpretation of muscle damage markers, age-related skeletal muscle fiber type changes, and skeletal muscle repair processes impaired by age-related immune system changes. All of these changes conspire to impair repair and replenishment of aged skeletal muscle post exercise. Therefore, this review compares and contrasts age-related recovery processes and intervention results following strenuous exercises.

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Chapter 1: Masters Recovery Overview

INTRODUCTION

Despite known age-related physical capacity reductions affecting aerobic, anaerobic, exercise efficiency, strength and power due to declines within the metabolic cardiovascular, and hormonal systems,[2] masters athletic participation and performance have been on the rise for several decades across classic "Olympic" and newly added events.[1] Compared with younger athletic events, the basis of masters athletic competition remains unchanged requiring high aerobic and anaerobic capacities, as well as, appropriately strong and powerful muscle contractions tuned for the particular event.[2] Therefore, describing recovery processes for this athlete group is important to aid achieving complete muscle repair and replenishment and improving subsequent performances resulting from training adaptations.

The focus of this review is to understand skeletal muscle recovery from mechanical and physiological damage. Mechanical damage alters the muscle physical structure causing disruption of the sarcomeres in myofibrils, Z-line streaming, and T-tubule damage resulting in measurable loss of maximal concentric contraction force.[3][4]Physiological damage alters the energy metabolism pathways, inflammation response pathways, nutrient delivery system, and neurological factors that delay recovery. There are interactions between these two types of damage to cause delayed neurological recovery due to mechanical damage.[5][62]The goal is to clarify recovery from exercise-induced muscle damage for aged and masters athletes. Present studies investigating the mechanisms of fatigue and delayed recovery responding to endurance activities have used resistance and high intensity test bouts to analyze the muscle recovery processes, and this review will examine their results. There remains a paucity

of study in some areas or recently published reviews that capture a majority of the relevant contributions in an area, and therefore, single studies will comprise many references used here.

BACKGROUND

The term "masters" has been defined as athletes who are older than the recognized peak age for a particular sport – typically between 30 and 40 years old.[6] The generally accepted notion that aging determines the decline in performance might instead be the result of increased sedentary life style, because research using older sedentary subjects has shown consistently improved physical capacities, and decline in physiological measures occurs more slowly in aged, recreational athlete populations than typically occur in the wider population. For example, male and female master athletes competing in the 2014 Pan Pacific Masters Games were shown to exhibit significantly better anthropometric, functional, and health characteristics compared with age-matched and generally less active [Australian] population.[8]

However, compared with sedentary adults, masters athletes of both sexes show greater absolute VO_2max decline with age possibly due to previous exercise lifestyle followed by reductions in habitual exercise.[2][7] However, aged and master athletes can maintain proficient exercise capabilities with continued training,[7] and therefore the notion of "use it or lose it" seems to apply to aging athletic performance if not the entire aging population as a whole.[10][11]

While aging processes obscure the study of recovery, some theorize that study of recovery in masters athletes aids in understanding the aging processes in general because it unmasks the muddle caused by age-related disease process interacting with aging processes.[8][9][13]

Brief Physiology review of Aged Athletes

Well known are the age-related decline in VO_2max. Athletic performance declines exponentially beginning around the seventh to eighth decade of life, and this age-related decline in athletic performance is typically greater in women compared with men.[7]

Gender Differences – A Brief Summary

Sex Hormone Effects

Muscle mass averages 36% higher in men than in women with more in the upper body (40%) versus lower body (33%). These differences exist from the infancy to old age and become most obvious after puberty. Sex hormones are implicated as the cause of the differences.[13] While estrogen seems to improve muscle contractile properties, its membrane stabilizing effects seem to resist muscle cell damage, and therefore, reduce enzyme leakage into blood plasma.[14] Therefore, studies relying on those blood markers of muscle damage must segregate reporting by gender. Studies have shown contrary results due in part to differences in participant age, fitness level, exercise protocols and exercise intensity, and comparisons confounded by actions by other hormones.[15]

An example of unintended consequences was shown using muscle damaging downhill run test protocols. Some oral contraceptives (OC) seem to have no impact on recovery,[16] while new, 3-rd generation, monophasic oral contraceptives (OC) (i.e. low androgen progestin) caused hypertrophy (but not significantly) resulting from resistance training compared to non-OC users. The ethinyl estradiol in the OCs was possibly responsible for the hypertrophic effect of resistance training.[17]

Improved nutrient delivery and increased energy for muscle structural repair may result from improved vascularization and skeletal muscle mitochondrial function shown in premenopausal and recent postmenopausal subjects. [18]

High levels of estrogen attenuate inflammation that causes delayed onset muscle soreness (DOMS) after exercise,[19] and high estrogen (estradiol) levels reduce oxidative stress-induced muscle damage, and is possibly associated with satellite cell activation and proliferation in skeletal muscle.[15]

Blood Markers of Muscle Damage Effects

Women have lower serum creatine phosphate kinase (CK) activity than do men at rest, and in response to equivalent exercise bouts including muscle damaging eccentric exercise.[21] The normal range of serum CK in males is 39–308 IU/L and in females is 26–292 IU/L[22] indicating wide variation.

CK concentrations and DOMS (delayed onset muscle soreness) in sedentary male and female cohorts post muscle-damaging downhill running bouts were shown to be gender specific. Independent of menstrual phase, CK response recovers more quickly in women. Moreover, the CK and DOMS responses occur in concert in men, but not in women following downhill running. The DOMS response in women is prolonged, which the authors speculated that this effect "…may be influenced by menstrual phase with possible prolonged muscle pain sensitivity." [24]

Fatigue

Older adults and females experienced a greater fatigue resistance for a sustained maximal-effort isometric knee extensor contraction compared with younger adults and

males who experienced greater peripheral and central fatigue and greater peripheral fatigue, respectively.[25]

In general women display greater endurance and faster recovery than men. Men show faster twitch force generation and rate of relaxation when fatigued due to female muscle containing a larger proportion of type I fibers that are oxidative (slow) with higher endurance.[14]

Eccentric Exercise Repeated Bout Protective Effect

This non-estrogen related effect is in response to an initial eccentric (ECC) bout that relieves damage from follow-on eccentric bouts in the same muscle group. Since 1983, the effect has been shown in different human muscle groups, and is thought to be the result of interaction between various neural, connective tissue and cellular factors.[26]

A single exercise bout is shown to have a prophylactic effect on muscle soreness and serum enzyme responses that last approximately 2 weeks;[27] the greatest adaptation occurred with the first repeat bout.[28]

Chapter 2: Skeletal Muscle Recovery Processes Described and Measured

INTRODUCTION

Independent of age, recovery processes are healing processes to repair structural and oxidative damage sustained during an exercise bout; recovery processes also rejuvenate physiological systems depleted during a bout. The overall goal is to return both systems to their pre-exercise state, and then further extend the capabilities of each system in response to more strenuous cycles of the physical training model.[2]

Repairing generalized cellular damage has been recently modelled in three stages connected in a spiral cascade. The stages are linked and defined by different signaling pathways, and failure to complete any single stage induces different pathogenic disease manifestations due to incomplete repair. These mechanical repair processes involve stem cells for the particular tissue type, and skeletal muscle satellite cells respond to these signaling pathways.[29]

Aging is shown to blunt these processes; indeed, satellite cell (skeletal muscle stem cell) density has been shown to drop with age, which may be reversed to some extent by increased physical activity level.[30]

MASTERS RECOVERY STUDIES – A STARTING POINT

Different training loads and protocols impose wide variations in recovery times based on the training status, age, and gender of the study participants. Recovery can take from less than 24hrs in response to an acute training session to more than 28 days in response to an excessive, "over-reaching" training stimulus.[6] Psychological status has been shown to require consideration because it can reduce recovery from the previous exercise bout.[31]

The recovery of aged and master athletes from exercise-induced mechanical and physiological damage has been studied sporadically for decades without consensus due to equivocal results.[2] For example, results become skewed using sedentary subjects to model aged and trained muscle behavior, poor control for training status and training volume of the subjects, comparing concentric versus eccentric contraction results, using a single measure to evaluate severity of muscle damage, combining gender results, and possibly projecting results of trained and untrained animal to humans.[6] A recent review focused on cycling protocols (concentric contractions) and included mention of resistance protocols, (50% eccentric contractions) and concluded that due to limited body of research, age has no effect on recovery unless exercise-induced muscle damage delays neuromuscular function recovery.[2]

Importantly, the mention of neurological function refers to the cited 55km trail-run study featuring significant downhill running bouts – i.e. eccentric contractions spread over the distance approximating 50% the total distance. Endurance trained master athletes who were performance matched to a younger cohort showed significantly

Impairment

MVC (maximal voluntary-isometric contraction) strength at 24 hrs. post run, and this remained depressed, but not significantly, compared to the younger cohort through the 72hr recovery period studied.[5]

Also measured were peak muscle twitch torque and contraction time that indicate neuromuscular performance. The masters group showed delayed return to baseline that remained significantly depressed after 72hrs post exercise compared to 24hrs for the young cohort. Therefore, even if all other muscle remodeling and replenishment processes were complete, these masters would not have been able to train heavily, and possibly would require a gradual re-introduction to training.[5] There have been

apparently no other studies of this effect using similar duration and intensity, and the closest in terms of distance are those of marathon events[33][34] and IronMan® triathlon events[35] that did not directly investigate this behavior.

In contrast, neuromuscular interactions were recently studied using a seated, leg-press protocol, (resistance exercise, and therefore, half eccentric contractions) and endurance trained cyclists. The results, likely due to training status, and significantly shorter exercise duration, and lack of extensive muscle mechanical damage, showed there was no apparent age-related factor explaining the significant decrease in maximum voluntary torque (MVC) coupled to insignificant changes in M-wave and twitch neurological parameters.[32] in old verses young.

Notably, very few of the studies have featured uniform controls for pre-experiment nutrition status nor recovery feeding protocols. Therefore, the masters recovery differences in these studies could point to effects caused by varied availability of substrate and associated anabolic signaling required to complete recovery.

Masters athletes appear to maintain the glycogenesis capabilities of younger athletes if the exercise protocols feature mainly concentric contractions in accustomed endurance exercises in order to minimize mechanical damage to the exercised muscle.[36][67] Some recent masters studies have focused on muscle protein synthesis (MPS) recovery activities driven by feedings of carbohydrate (CHO) and protein (PRO) in response to muscle-damaging eccentric exercise protocols.[38][41][84]

In muscle recovery conditions, appropriately timed, post exercise recovery feedings of CHO and CHO+PRO accelerated glycogenesis.[38] For example, it has been shown that feeding of CHO+PRO immediately post exercise increases glycogen storage 2-4 times compared to CHO alone when measured after 40min post feeding. The CHO+PRO feeding accounted for approximately half of the glycogen stored during the

total 4hr recovery period measured.[38] However, glycogenesis recovery time increases significantly when exercise protocols feature significant eccentric contractions due to the associated muscle damage.[36][67]

Muscle protein synthesis (MPS) rates post exercise respond in a similar fashion to the glycogenesis process due to the anabolic effect of insulin in response to post exercise feedings of either CHO alone (36% increase has been shown) or supplementing with CHO+PRO, which has been shown to increase MPS three times above base levels and increase fat oxidation.[42]

Nonetheless, masters athletes suffer impaired MPS measured by fractional synthesis ratio (FSR) compared to young athlete despite equivalent CHO+PRO feedings.[37] Research has suggested that, after muscle-damaging endurance exercise, masters athletes require at least twice the protein ingestion to increase recuperative effect that still remains slower than younger athletes.[40] Despite extensive investigation into age-related anabolic resistance in sedentary aging populations, little is known about how anabolic resistance affects post-exercise muscle protein synthesis, and thus muscle remodeling in aging athletes.[39][41]

SKELETAL MUSCLE STEM CELL & SATELLITE CELL ACTIVITIES

Satellite cell (SC) are differentiated (adult) muscle stem cells responsible for skeletal muscle regeneration responding to muscle damage, over use, or tension in individual muscle fibers.[30] SC response is independent of muscle fiber type, and instead relies on enervation of the damaged fibers to determine the appropriate remodeling.[45]

Human muscle studies have shown the SC role in plasticity depends on the quantity of satellite cells, and their viability to proliferate.[45] Aged muscle has been

shown the have reduced SC populations that may have compromised macrophage activity which stimulates SC activation.[45]

Aging Effects

Age-related chronic inflammation blunts SC activity,[44] and deterioration of the immune system – so called "immuno-senescence" – negatively affects the specific macrophages responsible for SC activation. In response to muscle damage, specific acute (72hr) remodeling mechanisms upregulate pro-inflammatory microphages that secrete many compounds to prepare the damage site for repair including cytokines that upregulate SC proliferation activities.[46] Aged macrophages delay muscle repair and recovery.[45] Highlighting this relationship, SC appear to have regenerative capabilities in rodent models when implanted into individual young fibers with intact SC, and by whole muscle transplantation.[30]

Training, Fiber Type & Recruitment Effects

Endurance training for both aged men and women maintains the SC pool showing similar density in type I and type II fibers despite the age-related decline of type II fibers.[50] Fibers recruited more often contain more SC, and therefore, mixed fiber muscles will contain similar SC quantities in the constituent type I and type II recruited fibers.[47]

The age-related decline in type II fiber count seems to be a detraining effect that can be reversed following a progressive resistance training protocol.[51] Similarly, heavy resistance strength training (HRST) has been shown to stimulate SC absolute population and activity, as well as, increase mitochondria populations in the recruited type II fibers in both aged men and women.[52]

Protein Feeding Effects

It is well known that exercise upregulates SC activity, additionally, recent studies featuring PRO feedings (broad spectrum essential amino acid and high leucine content whey supplementation) were shown to stimulate SC activity.[48]

It has been shown recently as well, however, that similar whey feedings significantly affect only type I fibers subjected to concentric exercise. These same whey feedings raised type I SC content, but not significantly, in response to eccentric exercise. Type II muscle SC content remained essentially unchanged across contraction type and feedings.[49] There was no measurement to confirm the extent underlying muscle damage usually associated with delayed type II fiber recovery response to eccentric exercises.

NSAIDS & EIMD

Non-steroidal inflammatory drugs inhibit the pro-inflammatory cyclooxygenase (COX) pathways, which promote inflammation and pain associated with exercise induced muscle damage. Despite their widespread use by young adult athletes, recent studies have suggested their use might dampen anabolic signaling, and reduce muscle satellite cell activity following exercise thus possibly impairing strength and hypertrophy development during training. There have been no studies investigating these effects in older athletes.[1]

FATIGUE

Describing neuromuscular (NM) fatigue involves measuring changes in the central nervous system as well as muscle responses responding to exercise.[53] Fatigue blunts power production by reduced shortening velocity and peripheral damage will reduce muscle force (strength.)[56] For example, central fatigue leads to an inability to recruit all motor units at the rates necessary to deliver desired force or power.[56] Intensity

and duration of exercise on large mass muscle determines fatigue,[54] while high intensity exercise affects the periphery,[55] and moderate intensity exercise causes changes in both periphery and central systems.[56] Peripheral fatigue can substantially recover within minutes after a supramaximal exercise, while NM function recovers slower after prolonged, moderate-intensity exercise.[57]

Master athletes suffer with significantly higher perceived fatigue and reported soreness compared to younger subjects;[6][62] however, fatigue seems to abate responding to post-exercise PRO feedings in veteran triathlete studies.[41]

Chapter 3: Age-Related Skeletal Muscle Recovery

Introduction

The recovery processes are complex interactions of physiological, biochemical, hormonal, biomechanical, and psychological systems that are affected by age-related changes. Using young athletes as the baseline model, recovery is described by an acute phase immediately post exercise that is followed by a chronic phase that can last 72 hrs. or more determined by the extent of damage.[2]

Both endurance and resistance fatiguing bouts have been used to study glycogen replacement and muscle protein synthesis in young athletes resulting in standardized feeding recommendations for master athletes.[41][1][42] Recent masters recovery feeding studies, however, have identified significant differences for masters skeletal muscle repair and strength recovery in response to PRO supplementation.[37]

The current PRO recommended supplementation post-bout for young athletes is 20g PRO, which was determined as the amount that maximized muscle protein synthesis and initiated the beginning of amino acid oxidation.[37] I could find no study defining a similar onset for master athletes even in light of the interest in anabolic resistance. A recent study however showed the rate of FSR slowed (second derivative in calculus) across all PRO treatments, however, mixed muscle FSR responding to 35gm was over twice the 20gm rate using less than 2x the treatment amount. (The 10g treatment actually reduced post prandial FSR.) [69] This suggests a larger treatment is required to further increase masters FSR. Future study is required to determine the relationships between larger whey treatments and FSR in elderly and master athletes.

Aged and master athletes may benefit from innovative approaches involving ketone ester compounds to amplify glycogenesis beyond CHO+PRO post exercise

feedings,[65] and all athlete age groups can benefit from whole food consumption to maintain baseline nutrition status.[64]

Acute Phase – Resting Homeostasis and Glycogenesis

Briefly, the acute recovery phase consists mainly of rapidly returning the physiological systems back to near homeostatic resting values, a faster rate of muscle glucose uptake as a result of increased muscle insulin sensitivity, and causing improved glucose absorption and glycogenesis.[42] Protein also raises insulin sensitivity in skeletal muscle to increase protein synthesis and glycogen refueling to return muscles to a pre-exercise state.[2]

Glycogen Recovery – Acute Feeding of Carbohydrate (CHO) and Protein (PRO)

While steady state feedings of CHO and PRO meals maintain energy and homeostatic structural balance – so called nutrition status, acute post exercise feeding of either CHO or PRO substrate or in combination amplifies plasma insulin levels beyond levels attained by either substrate alone. Glycogen recovery processes are relatively slow processes, but feedings timed to maintain glucose plasma concentrations maintain insulin concentrations, and are shown to minimize glycogen recovery time.[42]

CHO quantities consumed must be sufficient to trigger a sufficient insulin response to drive faster glucose uptake enabled by increased insulin sensitivity of skeletal muscle and glucose transporter activity. Furthermore, insulin sensitivity can be maintained many hours post exercise if regular CHO feedings continue in two-hour increments.[42]

Combining PRO to these CHO feedings amplifies glycogen storage 38% over the first 4 hour period post exercise, and the quantity of both CHO and PRO drives this

response dispelling the notion that calories drive the response – specifically, 40 minutes into recovery, the glycogen recovery rate was shown to be twice the rate of iso-caloric CHO feedings, and four times faster than iso-carbohydrate feedings. However, CHO substrate availability appears to rate limit glycogen storage.[42]

Glycogen Recovery – Acute Feeding of Ketone Esters

Post exercise, human muscle glycogenesis has been shown to benefit from ketone ester supplementation containing an amount that mimics a week of total fasting. The ketone supplement administered alone was ineffective; however, ketone and glucose supplementation in combination significantly raised both insulin sensitivity and insulin responsiveness to enhance muscle glycogenesis.[65]

Ketone bodies are an alternative fuel source for the brain (they can pass the blood-brain barrier), as well as skeletal muscle. Plasma ketones concentrations can become the preferential fuel source during exercise thus sparing glycogen stores in the liver and skeletal muscle enhancing endurance performance and can blunt the physiological symptoms of "overreach" to improve exercise performance in a subsequent bout.[66]

Further study of ketone ester activities is required to determine the interaction of nutrient timing, as well as, determining whether this insulin effect similarly amplifies muscle protein synthesis (MPS) recovery.

Glycogen Recovery – Delayed by Damaging Eccentric Contraction

Eccentric exercise has been shown to impair glycogenesis, and the amount of muscle damage determines the lower rate of glycogenesis. For example, a study comparing post exercise glycogenesis rates responding to CHO supplementation with more than 10g/kg/day showed the concentric muscle cohort completely replenished

glycogen stores in 24hrs, while the eccentric muscle cohort (damage confirmed using multiple blood markers) glycogenesis declined even further 2hrs post feeding.[36]

Glycogen Recovery – GLUT-4 Disrupted Glucose and Training Status

Muscle damaging eccentric contractions delay glycogenesis for all age populations due to post exercise transient GLUT-4 decreases that could be the result of strong mechanical cell damage that is more apparent in master athletes' impaired glucogenesis compared to young athletes.[6]

GLUT-4 concentrations are highly responsive to exercise stimulus.[20] For example, young and old, male and female sedentary subjects significantly increased GLUT4 concentrations after 7 days of 1hr concentric exercise training (cycling) at the same relative exercise intensity (<75% VO2max.) This improved training status is likely the result of upregulation of GLUT-4 transcription and translocation of GLUT-4 to cell surface membranes.[20]

Glycogen Recovery - mRNA Effects

Muscle glycogen content influences regulation of gene transcription.[68] For example, comparing responses to low CHO and high CHO feedings, the high CHO cohort showed increased mRNA driven down-regulation of MPS-blocking metabolic genes.[68]

Chronic Phase - Muscle Remodeling and Rejuvenation Processes.

The chronic recovery phase may last up to 72hrs post-exercise, however, extreme intensity and duration in the bout can increase recovery time.[2]

Importantly, specific repair responses involve repair of disrupted calcium signaling to reverse blunted excitation-contraction coupling and inflammatory response. Inflammatory responses include activation of protein degradation pathways, as well as, satellite cell activation.[6]

Carbohydrate (CHO) and Protein (PRO)

Post exercise protein remodeling studies using active adult populations subjected to acute exercise bouts used acute resistance exercise protocols versus using "contact sport, middle-distance, or endurance exercise" [64]

Additionally, while most recent research focused on whey protein supplementation due to its strong leucine and EAA profile, the authors included a few supplemental studies using whole food protein sources like beef or soy.[71] Moreover, there is very little information regarding how endurance exercise intensity and duration impacts the ingested protein dose–response curves on the post-exercise MPS.[64]

We know CHO and PRO illicit strong insulin responses separately and are additive in combination; insulin blunts catabolic processes and is permissive to strong anabolism; however, the CHO dose must be sufficient to induce the insulin response. Furthermore, steady state feedings of CHO and PRO maintain baseline energy and homeostatic structural balance, whereas bout-related feeding of either substrate or in combination amplifies anabolic muscle protein synthesis processes, and provides nutrient substrates required for the process.[42]

Studies using young athletes show that exercise triggers MPS rates post exercise even from unfed states, and amplified MPS rates in young athletes occur in response to combined CHO and PRO feedings likely due to the resulting amplified insulin response that permits MPS.[42] Insulin has been shown to permit MPS if insulin concentrations

reach 10-15 mU/l. Only then will sufficient plasma EAA concentrations trigger MPS. Confirmation of this interaction was shown perhaps unintentionally underfeeding subjects with CHO (8 grams) that resulted in no MPS.[69]

Endurance athletes apparently require more PRO than resistance athletes when consuming an energy balanced diet.[70]

Essential amino acids (EAA), and in particular leucine, have been found to trigger MPS strongly and preferentially to other amino acids, moreover, leucine and EAA availability rate limit the anabolic response while non-essential amino acids do not,[64] particularly in the elderly.[72][73]

Compared to an equivalent control amino acid mixture dose, whey protein hydrolysate supplementation significantly upregulated the gene expression responsible for positive MPS transcription causing dose-dependent initiation of mRNA translation mediated primarily through the mTOR signaling pathway.[73]

Master athlete recovery studies [37] and sedentary aged subject studies [74] show similar results to high-leucine whey and CHO supplementation, however, the master athlete MPS rates were significantly lower than those of young athletes despite equivalent recovery feedings. Adding antioxidants to these supplements improves MPS measured by improved strength and reduced soreness.[75]

ANABOLIC RESISTANCE

Skeletal muscle protein remodeling in master athletes' skeletal muscle has been shown to be impaired,[39] and the importance of post-exercise protein feeding for endurance athletes is increasingly being acknowledged [40] with its well-known role in creating a positive net muscle protein balance post-exercise.[41]

Anabolic resistance is the reduced muscle protein synthesis response of aged muscle compared to young muscle when post-exercise PRO feedings deliver the same amount of PRO substrate.[1] None the less, post-exercise PRO dose recommendations for masters athletes remain the same as for young athletes, and therefore, masters athletes may benefit from higher doses of post-exercise dietary protein, with particular attention directed to the leucine content.[39][41][68]

NEW INVESTIGATION AREA – NUTRIENT ABSORPTION

Strenuous endurance exercise has been shown to cause GI distress in young athletes,[87] and was recently characterized by increased zonulin activity in serum. Zonulin is a marker of epithelial breakdown specifically in the gut.[76] I could find no similar study for aged or master athlete behaviors.

Diet appears to improve the epithelial boundary, and improve cardiovascular health [78][79][88] indicating a possible implication to participating in anabolic resistance. For example, studies involving young and aged subjects have screened for GI disease,[74] and other studies using young and aged subjects measured increased PRO levels in the gut despite serum increases without comment about the normalcy of the amounts.[69]

Chapter 4: Studies of Skeletal Muscle Recovery

Unless noted otherwise, and in order to save space and minimize reading fatigue, I will use the following shorthand conventions in this chapter only on the following citations:

Fell and Williams, 2008[6] becomes Fell
Easthope et al.,2010[5] becomes Easthope
Doering et al.,2016[37] becomes Doering
Hayashi, K., et al., In press 2019[86] becomes Hayashi

DAMAGING PROTOCOLS REQUIRED

Skeletal muscle is the engine for motion, and therefore, to study muscle recovery, the experiment protocol must first sufficiently damage muscle through exercise or physical impact to cause strong and measurable recovery. This means use eccentric contractions versus concentric contractions.[6] No matter the source of damage, muscle soreness and reduction in strength results throughout the healing process. Eccentric contraction protocol bouts cause the most damage by way of the muscle fibers lengthening while under load.[80]

Early recovery studies of masters showed little or no age-related effect on recovery measured by return to pre-study strength. This seems to be due to the exercise protocols not causing sufficient damage.[6] Running does cause eccentric contractions to cushion the foot strike load, and preload the muscle for the subsequent associated concentric contraction, referred to the "stretch shortening cycle," [81] whereas, cycling is wholly a concentric action.[6]

Exercise protocols in master studies began to use eccentric bouts to maximize muscle damage in order to differentiate recovery processes between concentric and

eccentric muscle contractions.[5] Follow on studies quickly added treadmill downhill running protocols to induce ECC efficiently and effectively.[37][39][86]

This protocol analysis will ignore a popular referenced study because, despite the distances involved, it lacked few eccentric segments, and did not measure markers for muscle damage. For example, the 10km triathlon run course featured two hilly segments with an overall elevation bump of 360m, which at the face of it, would likely not rise to the skeletal muscle damage level of the other studies. Moreover, no strength recovery differences between groups were measured.[82]

ANALYSIS OF PROTOCOLS – COMPARING ENDURANCE ECCENTRIC CONTRACTION RECOVERY STUDIES

Easthope

The master cohort (45yrs+/- and training matched to the young cohort) showed significantly degraded strength (MVC *vastus lateralus*) (compared to young (30yrs+/-) beginning at 24hrs, and sustained for the full 72hr recovery period (young recovered at about 24hrs.) Significant masters neurological degradation occurred. M-wave duration was significantly longer compared to young at 24hrs, but recovered to insignificant levels at 72hrs. However, compared to young peak twitch torque & contraction time, masters measures were significantly depressed at 48hrs and remained at 72hrs post-exercise. CK and LDH showed expected increases and time dynamics.

ANALYSIS & COMMENT

While cohort ages were not significantly different, the masters age was relatively young compared to other studies,[37][62][69] and yet the masters cohort exhibited much slower recovery times.

The purpose of the recovery sub-max cycling bouts was not explained, and seems to be active recovery that may or may not favor young athlete recovery.[83]

Moreover, there was no control for either recovery feedings or recovery activities by either cohort instead relying on the honor system, which perhaps lead to reduced master recovery during the 72hrs timeline due to habitual underfeeding.[70][37]

Doering

The significantly older and significantly fatter master triathlete cohort was not training matched to young, (VO2max significantly lower than young) and showed significantly reduced myofibrillar FSR compared with younger in response to significant eccentric contraction induced muscle damage. The 72hr recovery period included plasma enzyme marker measurements accompanied by intense cycling time trial training to evaluate muscle strength losses. Each cohort received controlled recovery feedings. Although not significant, there was a trend for masters to recover from cycling performance at slower rates compared with younger triathletes.

ANALYSIS & COMMENT

Training Status

VO2max declines approximately 1% per year after 30yrs. [7]

I will use this relationship to model comparisons of masters participant cadres across studies, especially when training status is self-reported. In this case, and perhaps obviously, the master athletes are indeed "highly trained." [Table 1]

Feeding

Based on the 3-day diet record pre-study, these masters seem to habitually underfeed PRO compared to the young cohort. Specifically, the master habitual protein intake was 1.7 +/- g/kg/day compared to 2.4 +/- g/kg/day for the young.[37][70]

Moreover, the authors state “all meal consumption was recorded” which begs the question whether all subjects ate all assigned food. Reduced substrate availability would impair recovery performance in both cohorts. The authors do not mention these results - specifically, which subjects failed to finish all meals and why was this allowed?

Finally, despite a detailed mention in the discussion section, the feeding protocol failed to test the notion that increased PRO consumption by master athletes post exercise would further improve MPS rates. Specific mention of PRO dosing in studies doubles the suggested 20g PRO amount for young athletes (which was defined as the onset of amino acid oxidation.) [37]

I could find no study defining the same onset for master athletes even in light of the interest in anabolic resistance. A recent study did show the rate of FSR slowed across all master PRO treatments, however, mixed muscle FSR response to 35gm PRO more than doubled the 20gm FSR while using less than 2x the treatment amount. (The 10g treatment actually reduced post prandial FSR.) [69] This means there remains additional rate increase available to drive more FSR activity.

Hayashi

The study found no differences between young and older, male and female active adults recovering from a downhill protocol evaluated by visual analog pain scores, maximal isometric torque strength, and plasma CK and Mb concentrations. Subjects were grouped into young-sedentary (YS), young-trained (YT), and significantly older-trained (OT) cohorts (Old sedentary were not tested due to treadmill safety concerns.) The estrogen effects of sparing muscle damage were controlled testing young female subjects in the early follicular stage while all aged females were post-menopausal.

Post-bout measurements, however, suggest an alternate interpretation of the data that aligns with previous study results. The significantly different training status between YT and OT cohorts measured by VO2max shown by OT significantly lower than YT. The YT value was similar to the YT cohort values in previous studies.[5][37] (Table 1) This indicates a possible training effect [21][3] may confer a comparatively greater ability to recover from exercise compared to OT. Moreover, the OT group has significantly higher body fat composition compared to YT, and was higher than the YS group, supporting the observation of the relative de-trained status of the OT group.

Pre-Bout VO2max Comparison

Hayashi, K et al.,(submitted to press 2019)					
	Young(S)	Young(T)	Old (T)	Delta	Notes
Age (yr)	27.5 ± 2.2	26.8 ± 1.8	57.5 ± 2.3*†	30.7	† Diff to Young(T) (P < 0.05)
Body fat, %	20.4 ± 1.7	17.7 ± 1.1	22.6 ± 4.5 †		* Diff to Young(S) (P < 0.05)
V˙O2peak (mL/kg/min)	39.9 ± 1.6	51.4 ± 1.4 *	40.8 ± 3.2 †	-21%	
		Tanaka & Seals (2008) Predicted		-30%	
			Expected VO2max	**35.9**	
Easthope et al., (2010)					
	Young(S)	Young	Master	Delta	
Age (years)	n/a	30.5 (7.0)	45.9 (5.9)	15.4	not sig
Body fat, %		n/a	n/a		
VO2 peak (ml/min/kg)		58.8 (6.5)	55.0 (5.8)	-7%	not sig
		Tanaka & Seals (2008) Predicted		-15%	
			Expected VO2max	**43.5**	
Doering et al., (2016(a))					
		Young	Old	Delta & %	
Age (yr)		27 (25–29)	53 (52–56)*	26	* sig different (P < 0.05)
Body fat, %	n/a	7.7 (6.8–9.6)	12.5 (8.6–16.7)*		* sig different (P < 0.05)
V˙O2peak(mL/kg/min)		63.4 (56.4–66.0)	56.3 (48–61)*	-11%	* sig different (P < 0.05)
		Tanaka & Seals (2008) Predicted		-26%	
			Expected VO2max	**46.9**	
Fell & Williams (2006)					
		Young(n=6)	Old (n=5)	Delta & %	
Age (yr)	n/a	24 (+/-5)	45 (+/-6)*	31	* sig different (P < 0.05)
Body fat, %		11.2 (+/-4.5)	11.6 (+/-3.2)		
V˙O2peak (mL/kg/min)		58.7 (+/-5))	57.3 (+/-4.3)	-2%	
		Tanaka & Seals (2008) Pre		-31%	
			Expected VO2max	**40.5**	

Table 1: Comparison of cross group relative fitness levels of participants. Yellow indicates % body fat, and green compares Old VO2max measured to predicted using the Tanaka & Seals (2008) 1% per year decline.

However, the OT measurements in the study suggest otherwise – that the study protocols appear to have not caused sufficient muscle damage in the OT group compared to YT.

For example, comparing muscle damage marker CK activity between YT and OT groups shows the YT peak (160% of baseline) serum CK (significant from baseline) developed in 24 hours post exercise. The OT concentration peaked as well at 24hrs but at 80% of baseline. This is unusual because while CK increase is not strongly correlated to the extent of muscle damage,[3] it means qualitatively OT may have suffered less muscle damage than YT. Other studies have shown that their respective OT groups display significantly higher CK measures compared young training matched group,[5] and young unmatched group.[37]

CK serum concentration also cleared over the same time course as Mb which is unusual. Mb serum concentrations should peak approximately 8hrs post-damaging exercise, and clear within 24hrs. [21] In contrast, responding to muscle-damaging exercise, CK should peak at 48hrs post-exercise and clear by 72hrs unless the bout was excessively intense.[21]

The significantly lower OT RPE scores at 30mins for the lower limbs indicate less perceived exertion compared to both young groups, and therefore OT experienced less exercise intensity which is shown to be one of the primary causes of muscle damage.[4]

The pain scale (author's Table 4) on all three muscle groups confirms YS sustained comparatively more muscle damage than either YT and OT, however, compared to YT, OT recovered faster suggesting less muscle damage. (The leg muscle scores of all groups remained significantly raised at 72hrs compared to baseline indicating delayed recovery in these muscles.

Range of motion measurements (author's Table 3) and associated pain scales (author's Figure 1) seem to support the notion of reduced OT muscle damage despite no cross-group differences. OT range (and YS behavior which seems similar) remained unchanged from baseline on knee extension and hip flexion, and pain scales for both

confirm this idea. However, hip extension range of motion measurements showed the opposite relationship indicated by significant OT (and similar YS) motion impairment compared to baseline, however there were no group differences in pain scale.

The MVC torque measures align with the CK measurements and time course. Reduced 90degree MVC indicated YT sustained more muscle damage compared to OT at 24hrs post-bout again indicating the opposite relationship shown in other studies, [5][37] and suggesting reduced OT muscle damage compared to YT.

At 24hrs, YT began a steady recovery rate to 72hrs, whereas, OT sustained continued muscle damage and slower recovery until 48hrs, and then matched the recovery rate of YT to 72hrs. However, MVC strength still remained lower than baseline compared to YT. YT 45degree MVC showed insignificant strength decline compared to baseline, while OT and YS showed significant strength decreases and similar recovery time course to 72hrs.

Conclusion

The study of exercise recovery for the aged athlete and elite master athlete group has been an ongoing field for decades despite confounding effects of the aging process obscuring recovery physiological behavior. The skeletal muscle recovery processes for this group have the same objectives as for younger groups, but there are subtle negative differences amplified by several factors - increased age-related detraining and sedentary behaviors affecting the training status of aged athletes, gender and associated hormonal changes affecting measurement and interpretation of muscle damage markers, age-related skeletal muscle fiber type changes, and skeletal muscle repair processes impaired by age-related immune system changes. These all conspire to impair repair and replenishment of aged skeletal muscle post exercise.

Works Cited

1 Clifford T. Nutritional and Pharmacological Interventions to Expedite Recovery Following Muscle Damaging Exercise in Older Adults: A Narrative Review of the Literature. *Journal of Aging and Physical Activity.* in press 2019.

2 Borges N, Reaburn P, Driller M, Argus C. Age-Related Changes in Performance and Recovery Kinetics in Masters Athletes: A Narrative Review. *Journal of Aging and Physical Activity*. 24: 149 -157, 2016.

3 Koch RJ, Pereira R, Machado M. The creatine kinase response to resistance exercise. *J Musculoskelet Neuronal Interact.* 14(1): 68-77, 2014.

4 Close GL, Kayani A, Vasilaki A, and McArdle A. Skeletal Muscle Damage with Exercise and Aging. *Sports Med.* 35 (5): 413-427, 2005.

5 Easthope CS, Hausswirth C, Louis J, Lepers R, Vercruyssen F, and Brisswalter J. Effects of a trail running competition on muscular performance and efficiency in well-trained young and master athletes. *Eur J Appl Physiol*. 110:1107–1116, 2010.

6 Fell J and Williams AD. The Effect of Aging on Skeletal-Muscle Recovery From Exercise: Possible Implications for Aging Athletes. *Journal of Aging and Physical Activity*, 16, 97-115, 2008.

7 Tanaka H and Seals DR. Endurance exercise performance in Masters athletes: age-associated changes and underlying physiological mechanisms. *J Physiol* 586.1: 55–63, 2008.

8 Fien, S., Climstein, M., Quilter, C., Buckley, G., Henwood, T., Grigg, J., and Keogh, J. Anthropometric, physical function and general health markers of Masters athletes: a cross-sectional study. *PeerJ* 5:e3768, 2017.

9 Wroblewski AP, Amati F, Smiley MA, Goodpaster B, and Wright V. Chronic exercise preserves lean muscle mass in masters athletes. *Phys Sportsmed.* 39(3):172-8, 2011.

10 Tanaka Hirofumi. Aging of Competitive Athletes. *Gerontology* 63:488, 2017.

11 Cobley, JN, Moult PR, Burniston JG, Morton JP, and Close GL. Exercise improves mitochondrial and redox-regulated stress responses in the elderly: better late than never! *Biogerontology* 16:249–264, 2015.

12 McKean KA, Manson NA, and Stanish WD. Musculoskeletal injury in the masters runners. *Clin J Sport Med*. 16(2):149-54, 2006.

13 Liao Y, Peng Z, Chen L, Zhang Y, Cheng Q, Nüssler AK, Bao W, Liu L, and Yang W. Views for Whey Protein and/or Resistance Training Against Age-related Sarcopenia. *Aging and Disease* 10[1]; 158-174, 2019.

14 Haizlip KM, Harrison BC, and Leinwand L. Sex-Based Differences in Skeletal Muscle Kinetics and Fiber-Type Composition. *Physiology (Bethesda).* 30(1): 30–39, 2015.

15 Haines M, McKinley-Barnard SK, Andre TL, Gann JJ, Hwang PS, and Willoughby DS. Skeletal Muscle Estrogen Receptor Activation in Response to Eccentric Exercise Up-Regulates Myogenic-Related Gene Expression Independent of Differing Serum Estradiol Levels Occurring during the Human Menstrual Cycle. *Journal of Sports Science and Medicine*. 17:31-39, 2018.

16 Enns DL and Tiidus PM. The Influence of Estrogen on Skeletal Muscle - Sex Matters. *Sports Med.* 40(1): 41-58, 2010.

17 Dalgaard LB, Dalgas U, Andersen JL, Rossen NB, Møller AB, Stødkilde-Jørgensen H, Jørgensen JO, Kovanen V, Couppé , Langberg H, Kjær H, and Hansen M. Influence of Oral Contraceptive Use on Adaptations to Resistance Training. *Frontiers in Physiology*. 10:(824), 2019.

18 Nyberg M, Egelund J, Mandrup CM, Andersen CB, Hansen KMBE1, Hergel IMF, Valbak-Andersen N, Frikke-Schmidt R, Stallknecht B , Bangsbo J1, and Hellsten Y. Leg vascular and skeletal muscle mitochondrial adaptations to aerobic high-intensity exercise training are enhanced in the early postmenopausal phase. *J Physiol*. 595.9: 2969–2983, 2017.

19 Williams T, Walz E, Lane AR, Pebole M, and Hackney AC. The effect of estrogen on muscle damage biomarkers following prolonged aerobic exercise in eumenorrheic women. *Biol Sport.* 32(3): 193–198, 2015.

20 Cox, JH, Cortright RN, Dohm GL, Houmard JA. Effect of aging on response to exercise training in humans: skeletal muscle GLUT-4 and insulin sensitivity. *J. Appl. Physiol.* 86(6): 2019–2025, 1999.

21 Noakes TD: Effect of Exercise on Serum Enzyme Activities in Humans. *Sports Medicine.* 4: 245-267, 1987.

22 Arulselvi S, Sukheeja D, Trikha V, Pandey AK, Albert V, Pandey RM. Evaluation of Serum Creatine Kinase and Urinary Myoglobin as Markers in Detecting Development of Acute Renal Failure in Severely Injured Trauma Patients. *ISRN Emergency Medicine*. 241036: 8, 2013.

23 Danielsson T, Carlsson J, Schreyer H, AhnesjoÈ J, Siethoff LT, Ragnarsson T , Tugetam A, and Bergman P. Blood biomarkers in male and female participants after an Ironman-distance triathlon. *PLoS ONE* 12(6): e0179324, 2017.

24 Oosthuyse T, and Bosch AN. The Effect of Gender and Menstrual Phase on Serum Creatine Kinase Activity and Muscle Soreness Following Downhill Running. *Antioxidants*. 6:16, 2017.

25 Solianik R, Kreivenaite L, Streckis V, Mickevic˘iene˙ D , and Skurvydas A. Effects of age and sex on fatigability and recovery from a sustained maximal isometric voluntary contraction. *Journal of Electromyography and Kinesiology* 32: 61–69, 2017.

26 McHugh MP, Declan AJ, Connolly, Eston RG, and Gleim GW. Exercise-Induced Muscle Damage and Potential Mechanisms for the Repeated Bout Effect. *Sports Med*. 27 (3):157-170, 1999.

27 Lavender AP and Nosaka K. Comparison between old and young men for changes in makers of muscle damage following voluntary eccentric exercise of the elbow flexors. *Appl. Physiol. Nutr. Metab*. 31: 218–225, 2006.

28 Hassan ES. Muscle damage and adaptation after the second bout of eccentric exercise of the knee extensors. *J Sports Med Phys Fitness.* 54(5): 644-50, 2014.

29 Naviaux RK: Incomplete Healing as a Cause of Aging: The Role of Mitochondria and the Cell Danger Response. *Biology*. 8: 27, 2019.

30 Garcia-Prat l, Sousa-Victor p, and Munoz-Canoves P. Functional dysregulation of stem cells during aging: a focus on skeletal muscle stem cells. *FEBS Journal.* 280: 4051–4062, 2013.

31 Wagstaff CR. Emotion regulation and sport performance. *J Sport Exerc Psychol*. 36(4):401-12, 2014.

32 Bieuzen F, Hausswirth C, Louis J, and Brisswalter J. Age-Related Changes in Neuromuscular Function and Performance following a High-Intensity Intermittent Task in Endurance-Trained Men. *Gerontology*. 56:66–72, 2010.

33 Nikolaidis PT, Alvero-Cruz JR, Villiger E, Rosemann T, and Knechtle B. The Age-Related Performance Decline in Marathon Running: The Paradigm of the Berlin Marathon. Int. J. Environ. Res. Public Health. 16: 2022, 2019.

34 Martin TG, Pata RW, D'Addario J, Yuknis L, Kingston R, Feinn R. Impact of age on haematological markers pre- and post-marathon running. *Journal of Sports Sciences.* 33:19, 1988-1997, 2015.

35 Lepers R and Stapley PJ. Master Athletes Are Extending the Limits of Human Endurance. *Front. Physiol*. 7:613, 2016.

36 Costill DL, Pascoe DD, Fink WJ, Robergs RA, Barr SI, and Pearson D. Impaired muscle glycogen resynthesis after eccentric exercise. *J. Appl. Physiol.* 69(l): 46-50, 1990.

37 Doering TM, Jenkins DG, Reaburn PR, Borges NR, Hohman E, and Phillips SM: Lower Integrated Muscle Protein Synthesis in Masters Compared with Younger Athletes. *Med. Sci. Sports Exerc.* 48[8]: 1613–1618, 2016.

38 Ivy, J L. Goforth HW, Damon BM, McCauley TR, Parsons EC, and Price TB. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. *J Appl Physiol.* 93: 1337–1344, 2002

39 Doering TM, Reaburn PR, Phillips SM, and Jenkins DG. Postexercise Dietary Protein Strategies to Maximize Skeletal Muscle Repair and Remodeling in Masters Endurance Athletes: A Review. *International Journal of Sport Nutrition and Exercise Metabolism.* 26: 168 -178, 2016.

40 Moore DR, Camera DM, Areta JL, and Hawley JA. Beyond muscle hypertrophy: why dietary protein is important for endurance athletes. *Appl. Physiol. Nutr. Metab.* 39: 987–997, 2014.

41 Doering TM, Reaburn PR, Borges NR, Cox GR, and Jenkins DG. The Effect of Higher Than Recommended Protein Feedings Post-Exercise on Recovery Following Downhill Running in Masters Triathletes. *International Journal of Sport Nutrition and Exercise Metabolism*. 27: 76-82, 2017.

42 Ivy, JL. Regulation of Muscle Glycogen Repletion, Muscle Protein Synthesis, and Repair Following Exercise. *Journal of Sports Science and Medicine*. 3: 131-138, 2004.

43 Thornell LE, Lindstrom M, Renault V, Mouly V, and Butler-Brown GS. Satellite cells and training in the elderly. *Scand J Med Sci Sports*: 13: 48-55, 2003.

44 Renault V, Thorne LT, Eriksson PO, Butler-Browne G, and Mouly V. Regenerative potential of human skeletal muscle during aging. *Aging Cell.* 1: 132–139 2002.

45 Sorensen JR, Kaluhiokalani JP, Hafen PS, Deyhle MR, Parcell AC, and Hyldahl RD. An altered response in macrophage phenotype following damage in aged human skeletal muscle: implications for skeletal muscle repair. *FASEB J.* 33, 000–000, 2019.

46 Snijders T, Nederveen JP, McKay BR, Joanisse S, Verdijk LB, van Loon LJC, and Parise G. Satellite cells in human skeletal muscle plasticity. *Front. Physiol*. 6:283, 2015.

47 Gibson MC and Schultz E. Age-Related Differences in Absolute Numbers of Skeletal Muscle Satellite Cells. *Muscle & Nerve*. 6:574-580, 1983.

48 Reidy PT, Fry CS, Dickinson JM, Drummond MJ, and Rasmussen BB. Post-exercise essential amino acid supplementation amplifies skeletal muscle satellite cell proliferation in older men 24 hours postexercise. *Physiol Rep*, 5 (11), e13269, 2017.

49 Farup J, Rahbek SK, Knudsen IS, de Paoli F, Mackey AL, and Vissing K: Whey protein supplementation accelerates satellite cell proliferation during recovery from eccentric exercise. *Amino Acids.* 46:2503–2516, 2014.

50 Mackey AL, Karlsen A, Couppe C, Mikkelsen UR, Nielsen RH, Magnusson SP, and Kjaer M. Differential satellite cell density of type I and II fibres with lifelong endurance running in old men. *Acta Physiol*, 210: 612–627, 2014.

51 Verdijk LB, Snijders T, Drost M, Delhaas T, Kadi F, and van Loon LJC. Satellite cells in human skeletal muscle; from birth to old age. *AGE.* 36:545–557, 2014.

52 Roth SM, Martel GF, Ivey FM, Lemmer JT, Tracy BK, Metter EJ, Hurley BF, and Rogers MA. Skeletal Muscle Satellite Cell Characteristics in Young and Older Men and Women After Heavy Resistance Strength Training. *Journal of Gerontology*. 56[6]: B240–B247, 2001.

53 Giboin LS, Amiri E, Bertschinger R, and Gruber M. Active recovery affects the recovery of the corticospinal system but not of muscle contractile properties. *PLoS ONE*. 13(5):e0197339. 2018.

54 Marshall PWM, Finn HT, and Siegler JC. The Magnitude of Peripheral Muscle Fatigue Induced by High and Low Intensity Single-Joint Exercise Does Not Lead to Central Motor Output Reductions in Resistance Trained Men. *PLoS ONE.* 10(10): e0140108, 2015.

55 Carroll TJ, Taylor JL, and Gandevia SC. Recovery of central and peripheral neuromuscular fatigue after exercise. *J Appl Physiol.* 122: 1068–1076, 2017.

56 Allen DG, Lamb GD, and Westerblad H. Skeletal Muscle Fatigue: Cellular Mechanisms. *American Physiology Review.* 88:287–332, 2008.

57 Krueger RL, Aboodarda SJ, Jaimes LM, Samozino P, and Millet GY. Cycling Performed on an Innovative Ergometer at Different Intensities-Durations in Men: Neuromuscular Fatigue and Recovery Kinetics. *Appl. Physiol. Nutr. Metab*. Accepted Manuscript, 2019.

58 Brancaccio P, Lippi G, and Maffulli N. Biochemical markers of muscular damage. *Clin Chem Lab Med*. 48(6):757–767, 2010.

59 Amat AM, Corrales JA, Serrano FR, Boulaiz H, Salazar JC, Contreras FH, Perez OC, Delgado EC, Martın I, and Jimenez AA. Role of a-actin in muscle damage of injured athletes in comparison with traditional markers. *Br J Sports Med*. 41:442–446, 2006.

60 Callegari GA, Novaes JS, Neto GR, Dias I, Garrido ND, and Dani C. Creatine Kinase and Lactate Dehydrogenase Responses After Different Resistance and Aerobic Exercise Protocols. *Journal of Human Kinetics.* 58:65-72, 2017.

61 Berridge BR, Van Vleet J, and Herman E. *Haschek and Rousseaux's Handbook of Toxicologic Pathology* (Third Edition), 2013 Chapter 46 - Cardiac, Vascular, and Skeletal Muscle Systems.

62 Borges NR, Reaburn PR, Doering TM, Argus CK, and Driller MW. Age-related changes in physical and perceptual markers of recovery following high-intensity interval cycle exercise. *Experimental Aging Research*, 44:4, 338-349, 2018.

63 Ivy JL, Ding Z, Hwang H, Cialdella-Kam LC, Morrison PJ. Post exercise carbohydrate–protein supplementation: phosphorylation of muscle proteins involved in glycogen synthesis and protein translation. *Amino Acids*. 35: 89–97, 2008.

64 van Vliet S, Beals JW, Martinez IG, Skinner SK, and Burd NA. Achieving Optimal Post-Exercise Muscle Protein Remodeling in Physically Active Adults through Whole Food Consumption. *Nutrients.* 10:224, 2018.

65 Holdsworth, DA, Cox PJ, Kirk T, Stradling H, Impey SG, and Clarke K. A Ketone Ester Drink Increases Postexercise Muscle Glycogen Synthesis in Humans. *Med. Sci. Sports Exerc.*, [49] 9: 1789–1795, 2017.

66 Poffe C, Ramaekers M, Van Thienen R, and Hespel P. Ketone ester supplementation blunts overreaching symptoms during endurance training overload. *J Physiol.* 597.12: 3009–3027 2019.

67 Zehnder M, Muelli M, Buchli R, Kuehne G, and Boutellier B. Further glycogen decrease during early recovery after eccentric exercise despite a high carbohydrate intake. *Eur J Nutr.* 43:148–159, 2004.

68 Pilegaard H, Osada T, Andersen LT, Helge JW, Saltin B, and Neufer PD. Substrate availability and transcriptional regulation of metabolic genes in human skeletal muscle during recovery from exercise. *Metabolism Clinical and Experimental.* 54:1048–1055, 2005.

69 Pennings B, Groen B, de Lange A, Gijsen AP, Zorenc AH, Senden JMG, and van Loon LJC. Amino acid absorption and subsequent muscle protein accretion following graded intakes of whey protein in elderly men. *Am J Physiol endocrinol Metab.* 302: E992–E999, 2012.

70 Kato H, Suzuki K, Bannai M, and Moore DR: Protein Requirements Are Elevated in Endurance Athletes after Exercise as Determined by the Indicator Amino Acid Oxidation Method. *PLoS ONE.* 11(6): e0157406, 2016.

71 Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, and Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *J Appl Physiol.* 107: 987–992, 2009.

72 Katsanos KS, Kobayashi H, Sheffield-Moore M, Aarsland A, and Wolfe RR. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab.* 291: E381–E387, 2006.

73 Rieu I, Balage M, Sornet C, Giraudet C, Pujos E, Laurent J, Mosoni G, and Dardevet D. Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia. *J Physiol.* 575.1: 305–315, 2006.

74 Bell KE, Brook MS, Snijders T, Kumbhare D, Parise G, Smith K, Atherton PJ, and Phillips SM. Integrated Myofibrillar Protein Synthesis in Recovery From Unaccustomed and Accustomed Resistance Exercise With and Without Multi-ingredient Supplementation in Overweight Older Men. *Frontiers in Nutrition* 6:40, 2019.

75 Ives SJ, Bloom S, Matias A, Morrow N, Martins N, Roh Y, Ebenstein D, O'Brien G, Escudero D, Brito K, Glickman L, Connelly S, and Arciero PJ. Effects of a combined protein and antioxidant supplement on recovery of muscle function and soreness following eccentric exercise. *Journal of the International Society of Sports Nutrition* 14:21, 2017.

76 Tota Ł, Piotrowska A, Pałka T, Morawska M, Mikulˇa´kova´ W, Mucha D., Żmuda-Pałka M, and Pilch W. Muscle and intestinal damage in triathletes. *PLoS One.* 8:14(1):e0210651, 2019.

77 Fell J, Haseler L, Gaffney P, Reaburn P, and Harrison G. Performance during consecutive days of laboratory time-trials in young and veteran cyclists. *Journal of Sports Medicine and Physical Fitness*. 46:3:395-402, 2006.

78 Gundry SR. Abstract 137: Reversal of Endothelial Dysfunction Using Polyphenol Rich Foods and Supplements Coupled with Avoidance of Major Dietary Lectins. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 33:A137, 2013.

79 Gundry SR. Abstract 309: Twelve Year Follow up for Managing Coronary Artery Disease Using a Nutrigenomics Based Diet and Supplement Program With Quarterly Assessment of Biomarkers. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 35:A309, 2015.

80 Manfredi TG, Fielding RA, O'Reilly KP, Meredith CN, Lee HY, and Evans WJ. Plasma creatine kinase activity and exercise-induced muscle damage in older men. *Med. Sci. Sports Exerc.* 23[9]:1028-1034, 1991.

81 Nicol C, Avela J, and Komi PV. The Stretch-Shortening Cycle - A Model to Study Naturally Occurring Neuromuscular Fatigue. *Sports Med* 36 (11): 977-999, 2006.

82 Sultana F, Abbiss CR, Louis J, Bernard T, Hausswirth C, and Brisswalter J. Age-related changes in cardio-respiratory responses and muscular performance following an Olympic triathlon in well-trained triathletes. *Eur J Appl Physiol.* 112:1549–1556, 2011.

83 Kang MS, Kim J, and Lee J. Effect of different muscle contraction interventions using an isokinetic dynamometer on muscle recovery following muscle injury. *Journal of Exercise Rehabilitation.* 14(6):1080-1084, 2018.

84 O'Bryan KR, Doering TM, Morton RW. Do multi-ingredient protein supplements augment resistance training-induced gains in skeletal muscle mass and strength? A systematic review and meta-analysis of 35 trials. *Br J Sports Med.* Epub ahead of print: 2018.

85 Tanaka Hirofumi , Kevin D. Monahan, Douglas R. Seals, PHD: Age-Predicted Maximal Heart Rate Revisited . *Journal of the American College of Cardiology* 37[1], 2001.

86 Hayashi K, Pearman ME, Roy SJ, Laosiripisan J, Pasha EP, and Tanaka H. Recovery from strenuous downhill running in young and older physically active adults. *International Journal of Sports Medicine*. In press. 2019.

87 Pals, K L, Chang RT, Ryan AJ, and Gisolfi CV. Effect of running intensity on intestinal permeability. *J. Appl. Physiol*. 82(2): 571–576, 1997.

88 Gundry SR: Abstract 412: Dietary Lectins Cause Coronary Artery Disease via an Autoimmune Endothelial Attack Mediated by Interleukin 16. *Arteriosclerosis, Thrombosis, and Vascular Biology*.38:A412, 2018.

89 Huang WC, Chang YC, Chen YM, Hsu YJ, Huang CC, Kan NW and Chen SS. Whey Protein Improves Marathon-Induced Injury and Exercise Performance in Elite Track Runners. *Int J Med Sci.*14(7): 648–654, 2017.